REMARKS

Reconsideration of the above-identified application in view of the above amendment and the remarks below is respectfully requested.

Claim 35 has been canceled in this paper. Claims 1, 9, 16, 20-21, and 36 have been amended in this paper. No new claims have been added in this paper. Therefore, claims 1-12, 14-18, 20-23, 25-26, 30-34, 36 and 41 are pending and are under active consideration.

Claims 1-12, 14-18, 20-23, 25-26, 30-36 and 41 stand rejected under 35 U.S.C. 112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." In support of the rejection, the Patent Office states the following:

> Claims 9, 16, 21, and 36 are indefinite for recitation of the phrase "suited automate." It is not clear what the phrase means because it has not generally recognized meaning and it is not defined in the specification. Although the specification provides examples of a suited automate on page 24 of the substitute specification filed 30 May 2002, a definition of the metes and bounds of the phrase is absent, rendering the phrase indefinite.
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> Claim 35 is indefinite because step d of claim 1 requires preceding step c for antecedent basis for a first knowledge base.
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> Claims 1-12, 14-18, 20-23, 25, 26, 30-36, and 41 recite the limitation "the genes exhibiting a different level of cytosine methylation" in claim 1, step e. There is insufficient antecedent basis for this limitation in the claim.

Applicants respectfully traverse the subject rejection. Insofar as the rejection is based on the use of the term "suited automate" in claims 9, 16, 21 and 36, this basis for the rejection has been

removed as claims 9, 16, 21 and 36 no longer recite this term. Insofar as the rejection is based on claim 35, this basis for the rejection has been removed as claim 35 has been canceled herein. Insofar as the rejection is based on an alleged lack of antecedent basis for the expression "the genes exhibiting a different level of cytosine methylation" in claim 1, step e, Applicants have amended claim 1 to overcome this basis for the rejection.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1-8, 10-11, 14-15, 17-18, 20, 25-26, 30-31 and 41 stand rejected under 35 U.S.C. 102(b) "as being anticipated by Kikyo et al. in light of New England Biolabs and Siegfried et al." In support of the rejection, the Patent Office states the following:

> The claims are drawn to a method of determining differential gene expression, and analyzing methylation states of the gene. In some embodiments the gene expression is determined from a biological tissue, gene expression of healthy and diseased individuals are compared, gene expression is determined by analysis of mRNA, differential display is used to compare gene expression, expression levels of 100 genes are analyzed, and the methylation state is measured enzymatically. Claim 41 is drawn to devices used in the method.
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> Kikyo et al. shows in the abstract and throughout a method of analysis of mouse embryo tissue for genes that are differentially expressed between normal embryos and abnormal embryos with chromosomal translocations. A differentially expressed neuronatin (Nnat) gene was shown to be imprinted by methylation analysis. Kikyo et al. shows on pages 68-69 differential display analysis of mRNA from the embryos, in which eighty primer pairs were used, and approximately 80-100 bands per primer pair were observed. Ten differentially expressed bands corresponding to differentially expressed genes were observed. Two genes were identified as H19 and Nnat (see figures 1A and 1B). Kikyo et al. further noted on page 69 prior art that used subtraction hybridization to identify Nnat as a differentially expressed gene, and verified Nnat differential

expression by a reverse transcriptase-polymerase chain reaction method (see figure 1C). Kikyo et al. subsequently analyzed the Nnat gene for methylation by digestion with a panel of restriction endonucleases Hind III, BssH II, Eag I, and Sac II (see figure 6).

The New England Biolab website establishes that BssH II, Eag I and Sac II enzymes are inhibited by methylation at CpG sites.

Siegfried et al. establishes on page R305 that CpG methylation is a term of art meaning that a cytosine is methylated.

Applicants respectfully traverse the subject rejection. Claim 1, from which claims 2-8, 10-11, 14-15, 17-18, 20, 25-26, 30-31 and 41, has been amended herein and now recites "[a] method for the development of gene panels for diagnostic and therapeutic purposes, comprising the steps of:

a) isolating at least one biological sample from each of at least two groups of biological material containing mRNA and/or proteins;

b) analysing the expression level of at least one gene in at least one of the biological samples;

c) selecting the gene(s) exhibiting a different expression level between said at least two groups of biological material, whereby a first knowledge base is generated;

d) analysing the level of cytosine methylation of at least one gene of said first knowledge base in at least one of the biological samples of step a) by means comprising treatment with bisulphite, hydrogen sulphite or disulphite;

e) selecting gene(s) exhibiting a different level of cytosine methylation between said at least two groups of biological material, whereby a second knowledge base is generated; and

f) adding selected genes from the second knowledge base to a gene panel."

Claim 1 is not anticipated by Kikyo et al. in light of New England Biolabs and Siegfried et al. for at least the reason that Kikyo et al. does not teach or suggest, among other things, the claimed step of analyzing the level of cytosine methylation by means comprising treatment with bisulphite, hydrogen sulphite or disulphite.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1, 22-23 and 32-34 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Kikyo et al. as evidenced by New England Biolabs and Siegfried et al." In support of the rejection, the Patent Office states the following:

> The claims are drawn to repeating the method of claim 1 which is drawn to a method of determining differential gene expression, and analyzing methylation states of the gene.
>
> Kikyo et al. shows in the abstract and throughout a method of analysis of mouse embryo tissue for genes that are differentially expressed between normal embryos and abnormal embryos with chromosomal translocations. A differentially expressed neuronatin (Nnat) gene was shown to be imprinted by methylation analysis. Kikyo et al. shows on pages 68-69 differential display analysis of mRNA from the embryos, in which eighty primer pairs were used, and approximately 80-100 bands per primer pair were observed. Ten differentially expressed bands corresponding to differentially expressed genes were observed. Two genes were identified as H19 and Nnat (see figures 1A and 1B). Kikyo et al. further noted on page 69 prior art that used subtraction hybridization to identify Nnat as a differentially expressed gene, and verified Nnat differential expression by a reverse transcriptase-polymerase chain reaction method (see figure 1C). Kikyo et al. subsequently analyzed the Nnat gene for methylation by digestion with a panel of restriction endonucleases Hind III, BssH II, Eag I, and Sac II (see figure 6).
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> The New England Biolab website establishes that BssH II, Eag I and Sac II enzymes are inhibited by methylation at CpG sites.

> Siegfried et al. establishes on page R305 that CpG methylation is a term of art meaning that a cytosine is methylated.
>
> Kikyo et al. does not show repetition of steps.
>
> It would have been obvious to a person of ordinary skill in the art at the time the invention was made to repeat the steps of Kikyo et al. for the purpose of analysis of additional tissue and genes for determination of correlations between expression and methylation, as shown by Kikyo et al.

Applicants respectfully traverse the subject rejection. As noted above, claim 1 is patentable over Kikyo et al., New England Biolabs and Siegfried et al. for at least the reason that these references, taken alone or in combination, do not teach or suggest a method that comprises the step of analyzing the level of cytosine methylation by means comprising treatment with bisulphite, hydrogen sulphite or disulphite. Claims 22-23 and 32-34, which depend from claim 1 and recite additional features thereto, are patentable over the applied combination of references for at least the same reasons as claim 1.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1 and 12 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Kikyo et al. in view of Anderson et al." In support of the rejection, the Patent Office states the following:

> The claims are drawn to the method of claim 1 which is drawn to a method of determining differential gene expression, and analyzing methylation states of the gene with the further limitation that both mRNA and protein levels are measured.
>
> Kikyo et al. shows in the abstract and throughout a method of analysis of mouse embryo tissue for genes that are differentially expressed between normal embryos and abnormal embryos with chromosomal translocations. A differentially expressed neuronatin (Nnat) gene was shown to be imprinted by methylation analysis.

Kikyo et al. shows on pages 68-69 differential display analysis of mRNA from the embryos, in which eighty primer pairs were used, and approximately 80-100 bands per primer pair were observed. Ten differentially expressed bands corresponding to differentially expressed genes were observed. Two genes were identified as H19 and Nnat (see figures 1A and 1B). Kikyo et al. further noted on page 69 prior art that used subtraction hybridization to identify Nnat as a differentially expressed gene, and verified Nnat differential expression by a reverse transcriptase-polymerase chain reaction method (see figure 1C). Kikyo et al. subsequently analyzed the Nnat gene for methylation by digestion with a panel of restriction endonucleases Hind III, BssH II, Eag I, and Sac II (see figure 6).

The New England Biolab website establishes that BssH II, Eag I and Sac II enzymes are inhibited by methylation at CpG sites.

Siegfried et al. establishes on page R305 that CpG methylation is a term of art meaning that a cytosine is methylated.

Kikyo et al. does not show measurement of protein levels.

Anderson et al. shows comparison of human liver gene expression by measurement of mRNA levels and corresponding protein levels (as measured by two-dimensional protein electrophoresis). Anderson et al. shows moderate levels of correlation between mRNA levels and protein levels in figures 1 and 2. Anderson et al. conclude on page 537 that determination of protein levels allows for a better understanding of multi-level gene expression control in complex organisms such as man.

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the method of Kikyo et al. by additional use of the protein analysis method of Anderson et al. because Anderson et al. shows that determination of correlations between mRNA and protein levels allows for better understanding of gene expression controls.

Applicants respectfully traverse the subject rejection. Claim 1, from which claim 12 depends, is patentable over Kikyo et al. for at least the reasons given above. Anderson et al. does not cure all

of the deficiencies of Kikyo et al. Therefore, claims 1 and 12 are patentable over the applied combination of Kikyo et al. and Anderson et al.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is

required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on July 3, 2006.

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Edward M. Kriegsman